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Growth, yield and aerenchyma formation of sweet and multipurpose sorghum (*Sorghum bicolor* L. Moench) as affected by flooding at different growth stages

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Abstract

The greenhouse experiment was a 4 × 2 factorial in a RCB design with four replications. Three flooding treatments were applied at the early vegetative stage (EV), early reproductive stage (ER) and mid reproductive stage (MR). A non-flooded control group was used. Two proposed bioenergy sorghum types studied were sweet sorghum (Wray) and multipurpose sorghum (SP1). The results showed that plant height, stem diameter, leaf area, leaf dry weight, shoot dry weight, primary root length and root dry weight of both cultivars were significantly reduced by flooding at EV and ER. However, there was no significant difference from the control at MR. Nodal root number were restricted when flooding was applied at EV but increased over the control at ER and MR in both cultivars. Root length and root dry weight, developed in water above soil surface, were significantly higher in Wray flooded at ER. In both cultivars, aerenchyma spaces were formed in the nodal and lateral roots of the flooded plants with the significantly highest number, forming during EV. Aerenchyma was more developed in roots, located above the soil, than in those located in the soil. There were more aerenchyma spaces in the sweet sorghum's roots and stalk bases than in the multipurpose sorghum. At harvest, it was found that flooding applied at EV and ER had significantly reduced the stalk yield of both cultivars. The Wray had been the least affected by flooding at MR. These findings suggest that both sorghum types are susceptible to flooding at EV. Judging from the recovery capacity of stem diameter and height at a later growth stage, sweet sorghum was more tolerant to flooding than multipurpose sorghum. Nodal root development and aerenchyma formation in roots and stalk bases may be important acclimation responses to flooding.

Keywords: Aerenchyma;Multipurpose sorghum; Nodal root; Sweet sorghum; Flooding.

Abbreviations: AC: aerenchyma; CK: control; Cv: cultivar; DAE: days after emergence; DEDE: Department of Alternative Energy Development and Efficiency Ministry; DW: dry weight; ER: early reproductive stage; EV: early vegetative stage; FAO: Food and Agriculture Organization; LA: leaf area; LR: lateral root; MR: mid reproductive stage; NR: nodal root; SP1:Supanburi 1.

Introduction

In Thailand, the use of ethanol for transportation is being promoted in alignment with the Thai government's objectives of reducing reliance on imported oil and to reduce greenhouse gas emissions. Thailand's ethanol production is projected to increase to 3.3 billion liters annually (DEDE, 2009a) by 2022. However, the quantities of current feedstock materials, such as cassava and sugarcane molasses, that are used to produce ethanol may not be sufficient to ensure a supply for future bioethanol demand (DEDE, 2009b). Sorghum could provide an additional source of ethanol that may be able to ensure sufficient future production of ethanol in Thailand.

Sorghum (*Sorghum bicolor* L. Moench) has aroused the interest of researchers throughout the world for its remarkable yield potential, which is present even in marginal environments (Mariani et al., 1989), as well as its potential for several non-food uses. It provides grain and stems for a feedstock for sugar, alcohol, syrup, fuel and paper as well as for animal feed (Doggett, 1988). In light of these facts, sorghum is now being developed as a potential bioenergy crop. Sweet sorghum, grain sorghum and fibre or new-specific high biomass cellulose sorghum have all been proven to be a promising bioenergy feedstock (Rooney et al., 2007; Jaradat, 2010). The optimum types of sorghum for biofuel production mainly depend on the type of conversion process used to extract ethanol. Energy production from sorghum has already been proven to economically attractive with high energy efficiency (Dolciotti et al., 1998; Reddy et al., 2005; Sakellariou-Makrantonaki et al., 2007). It is also environmentally safe (Barbanti et al., 2006) and positively affects greenhouse gas balance (FAO, 2008). However, when evaluating the potential of an energy crop, one must keep in mind its competition with crops grown for food. In Thailand, most arable land is occupied by staple crops, primarily rice, of which most farmers grow only a single crop annually in the mid-rainy season. The introduction of sorghum as a pre-rice crop has the potential to meet increasing demand for biomass feedstock without negatively impacting food crop production. This is also beneficial to farmers as they are able to earn more income. Nevertheless, due to current land management practices and variable precipitation, flooding is a major constraint on crop growth and yield in pre- rice crop

Table 1. ANOVA table of individual flooding treatment at different growth stages and cultivars of shoot and root growth.

Treatments	Shoot DW (g plant^{-1})	Height (cm)	Stem diameter (cm)	LA	Leaf DW (g plant^{-1})	Root DW (g plant^{-1})	Root length (m plant^{-1})	Root no. Per plant
EV (F)								
Control	23.89a	139.88a	1.52a	0.45a	12.93a	2.92a	167.00a	25.50
Flooding	4.15b	91.38b	0.26b	0.09b	2.40b	0.36b	42.84b	23.00
F-test	**	**	**	**	**	**	**	ns
Cultivar (C)								
Wray	13.54	119.25	1.26a	0.24	7.11	1.14b	56.56b	22.88
SP1	14.50	112.00	0.50b	0.30	8.21	2.13a	153.27a	25.63
F-test	ns	ns	**	ns	ns	**	**	ns
F × C								
F-test	ns	ns	*	*	ns	**	**	ns
ER (F)								
Control	111.76a	249.75a	1.93	0.59a	28.43a	13.58a	403.91a	45.38b
Flooding	87.54b	195.38b	1.80	0.45b	22.00b	4.24b	171.44b	72.38a
F-test	**	**	ns	**	**	**	**	**
Cultivar (C)								
Wray	105.93a	247.00a	2.20a	0.58a	27.42a	7.85b	255.71b	59.13
SP1	93.36b	198.13b	1.80b	0.46b	23.01b	9.97a	319.64a	58.63
F-test	*	**	**	**	*	*	*	ns
F × C								
F-test	ns	ns	ns	ns	ns	*	**	ns
MR (F)								
Control	184.56	254.25	2.01	0.56	28.35	17.90	566.06a	48.34b
Flooding	177.67	267.75	1.97	0.45	22.41	15.13	63.11b	69.00a
F-test	ns	ns	ns	ns	ns	ns	**	**
Cultivar (C)								
Wray	225.70a	293.75a	2.24a	0.60a	30.90a	16.78	218.82b	66.00a
SP1	136.53b	228.25b	1.74b	0.41b	19.87b	16.25	410.35a	51.44b
F-test	**	**	**	*	**	ns	**	**
F × C								
F-test	ns	*	ns	ns	ns	ns	**	ns

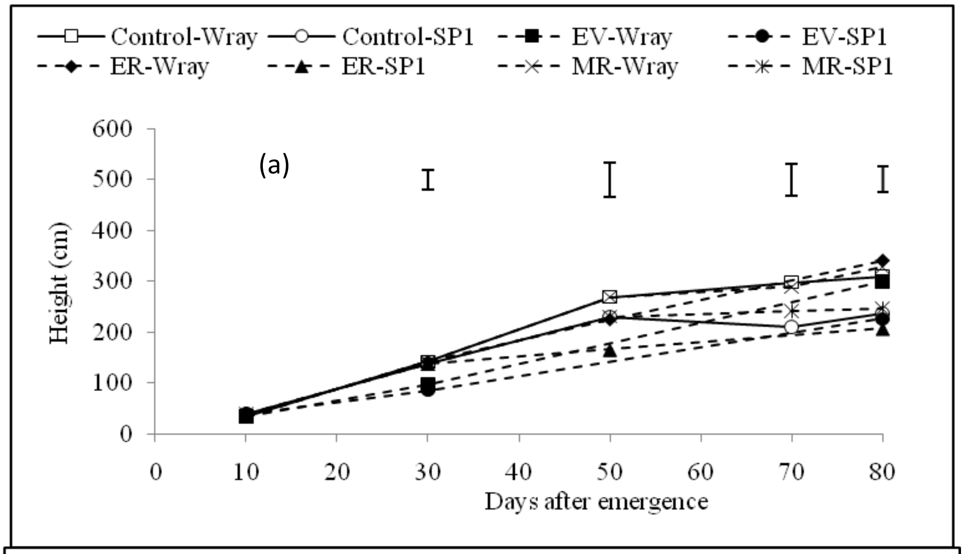
*,** Significant at P≤0.05 and 0.01 levels, respectively and ns = not significant. Flooding = control and flooding treatment, EV: early vegetative stage; ER: early reproductive stage; MR: mid reproductive stage and control: non- flooding. LA: leaf area and DW: dry weight

management (Polthanee, 1997). The ability to maintain production of sorghum under flooded conditions is necessary, but its response to flooding is not yet well understood. The evaluation of the effects of flooding on plant growth and yield parameters is an important factor in choosing the most suitable location for each variety of sorghum. Flooding causes a series of physiological, chemical and biological changes in soil (Zaidi et al., 2003), including inhibiting root and shoot growth, changing water and nutrient uptake, and altering physiological properties (Zhuo and Lin, 1995; Ahmed et al., 2002; Pang et al., 2004). Ultimately, flooding reduces plant yield (Orchard and Jessop, 1984; Umaharan et al., 1997; Zaidi et al., 2003; 2004). The adverse effects of flooding on plant growth and yield mostly depend on a plant's species or genotype (Orchard and Jessop, 1984; Umaharan et al., 1997; Pang et al., 2004; Zaidi et al., 2004) and the growth stage of that plant before flooding commences (Orchard and Jessop, 1984; Zhuo and Lin, 1995; Zaidi et al., 2004). Formation of aerenchyma in root cortex (McDonald et al., 2002; Zaidi et al., 2003; Pang et al., 2004) and the stalk (Glaz et al., 2004; Gilbert et al., 2007), as well as adventitious root formation (Pardales et al., 1991; Zaidi et al., 2004) have been widely accepted as adaptive/acclimation responses to flooding stress and have been found to be closely related to shoot growth and yield (Zaidi et al., 2004). The evaluation of flooding acclimation traits in both sorghum types could provide useful information for a flooding tolerance breeding program. Growth, yield and root response to waterlogging has been previously observed in grain sorghum (Orchard and Jessop, 1984; 1985; Pardales et al., 1991; McDonald et al., 2002). However, little is known about the response to flooding stress of sweet and multipurpose sorghum. And comparative studies between both sorghum types under anoxic conditions are rare. Therefore, the aim of this study was to investigate and compare the effects of flooding on plant growth and yield of sweet and multipurpose sorghum at different growth stages as well as to identify their acclimation traits to flooding stress.

Results

Shoot growth response

Plant height was significantly reduced to 35% and 22% lower than the control when 20 days of flooding was applied at the early vegetative stage (EV) and the early reproductive stage (ER), but plant height was not significantly different from the control when flooded at the mid reproductive stage (MR) (5% of the control) (Table 1). There was no significant difference between cultivars when flooded at EV, but significant differences were found at ER and MR (Table 1). The significant interaction of water regime × cultivar for height was noted when flooding was applied at MR. Cv. Wray under non-flooded and flooding conditions had similar height, while height was increased over control in cv. SP1 (Fig. 1a). At final sampling, the significant effects of flooding treatments, cultivars were found. Plants subjected to flooding at EV had lowest high, while plants subjected to flooding at ER and MR could recover to control levels (Table 2).There were significant water regime × cultivar interactions. Cv. SP1 flooded at ER had the lowest plant height (also lower than its



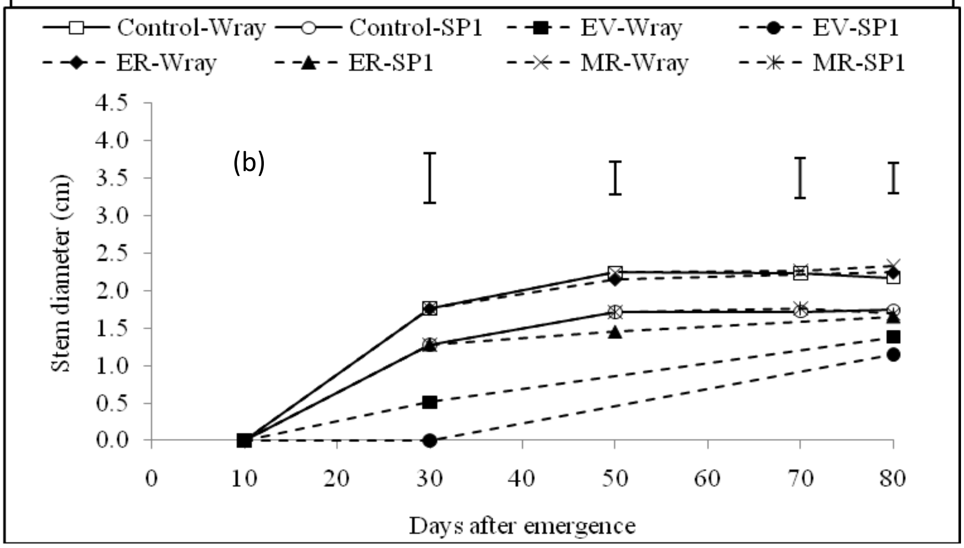


Fig 1. Change in plant height (a) and stem diameter (b) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV: early vegetative stage; ER: early reproductive stage; MR: mid reproductive stage and control: non-flooding.

respective control), while height was increased over its respective control in cv. Wray (Fig. 1a). Stem diameter was significantly reduced by flooding at EV (83%), but not at ER and MR. Cultivar effects were significant when water was applied at EV and ER but not at MR (Table 1). The significant water regime × cultivar interactions were found only when flooding was applied at EV and ER (Table 1). Cv. SP1 flooded at EV had the lowest stem diameter (Fig. 1b). At final sampling, adverse effects on stem diameter by flooding at EV persisted, resulting in a 38% reduction compared to the control but sorghum at ER and MR had stem diameter similar to those of the control. There were significant cultivar effects and water regime × cultivar interactions for stem diameter (Table 2). Damages from flooding at EV of cv. SP1 could not be reduced, whereas flooding at MR in cv. Wray increased stem diameter to more than that of the control (Fig.1b). LA and leaf dry weight were significantly lowest when flooding was applied at EV (80% and 81% reduction, respectively), while flooding at ER decreased these reductions (24% and 23%, respectively). Flooding at MR resulted in similar LA and leaf dry weight to the control (Table 1). Both cultivars had similar LA and leaf dry weight when flooded at EV but cv. Wray had significant higher leaf traits than cv. SP1 when flooded at ER and MR (Table 1). The significant water regime × cultivar interactions were noted for LA when flooding was applied at EV. Cv. SP1 had highest LA in the non-flooding treatment and lowest in the flooding treatment (Fig. 2a). During the recovery period, there was no significant difference among flooding treatments for LA. Significant cultivar effects and water regime × cultivar interactions were found in both leaf traits (Table 2). Leaf traits of cv. Wray flooded at MR were similar to its respective control. Cv. Wray flooded at EV and ER maintained their leaf traits better than the cv. SP1 did. LA (Fig. 2a) and leaf dry weight (Fig. 2b) were significantly lowest in cv. SP1 for all treatments (Fig. 2a and b). Shoot dry weight was significantly lowest when flooding at EV (83%), followed at ER (22%) but shoot dry weight was similar to the control when flooded at MR (Table 1). Both cultivars had similar response when subjected to flooding at EV. But cv. Wray had significantly higher shoot dry weight than cv. SP1 when flooded at ER and MR (Table 1). Nevertheless, there was no significant water regime × cultivar interaction.

Table 2. Effects of flooding treatments at different growth stages and cultivars on shoot growth and yield of sweet sorghum at final sampling (recovery).

Treatments	Shoot dry weight (g $plant^{-1}$)	Stalk yield (g $plant^{-1}$)	Height (cm)	Stem diameter (cm)	Leaf area (m^2 $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)
Flooding (F)						
EV	88.75c[1]	252.89c	249.25b	1.27b	0.32	13.62b
ER	119.22b	333.65b	258.63ab	1.94a	0.34	16.67ab
MR	175.35 a	452.52a	269.75a	2.01a	0.34	18.34a
Control	189.72a	489.11a	259.00ab	2.04a	0.35	19.01a
F-test	**	**	*	**	ns	**
Cultivar (C)						
Wray	167.87a	517.06a	290.31a	2.07a	0.47a	23.93a
SP1	118.66b	247.03b	228.00b	1.56b	0.20b	9.88b
F-test	**	**	**	**	**	**
F × C						
F-test	ns	**	**	**	**	**

*,** Significant at P≤0.05 and 0.01 levels, respectively and ns = not significant. [1]Means followed by the same letter are not significantly different.

At harvest, plants flooded at EV and ER could not completely recover from flooding damages, giving 53% and 37%, respectively, lower shoot dry weight than the control; however, plants flooded at MR had no significantly different shoot dry weight from the control. Cv. Wray showed significant higher shoot dry weight than cv. SP1 (Table 2). There was no significant water regime × cultivar interaction in shoot dry weight (Table 2 and Fig. 2c).

Primary root growth response

Twenty days of flooding significantly reduced root length and root dry weight in soil or primary root growth when sorghum was subjected to flooding at EV and ER. Root dry weight and root length were more severely reduced when flooded at EV, 88% and 74% respectively, and the reduction was decreased to 69% and 58% at ER. Flooding significantly reduced root length (89%) only when plants were subjected to flooding at MR (Table 1). There were significant cultivar effects on root traits when plants were subjected to flooding at EV and ER as well as for root length when flooding was applied at MR. Significant water regime × cultivar interactions were noted for root dry weight and root length when flooding was applied at EV and ER as well as for root length at MR (Table 1). Root length and root dry weight were highest in non-flooded SP1 at both growth stages, while flooded SP1 showed lowest root traits. Root length in mid-reproductive flooding treatment also showed a similar response to the previous growth stage (Fig. 3a). At recovery, plants flooded at EV had increased root length more than the plants flooded at MR had, while sorghum flooded at ER had root length similar to that of the control. Nevertheless, root dry weight of sorghum flooded at EV could not completely recover, while there was no significant difference from the control in other treatments (Table 3). Both cultivars had similar root lengths but significantly higher root dry weight was found in cv. Wray (Table 3). There were no significant water regime × cultivar interactions for root length (Fig. 3a) and root dry weight (Fig. 3b).

Root development in water

Flooding at ER and MR significantly increased newly-nodal root numbers to 7% and 43%, respectively but not at EV (Table 1). Both cultivars had similar nodal root development when flooded at EV and ER. Wray produced significantly higher nodal root numbers than did cv. SP1 at MR (Table 1). There was no significant water regime × cultivar interaction for nodal root numbers (Table 1 and Fig. 3c). At recovery, plants subjected to flooding at EV had significantly lowest nodal root numbers, while other treatments had root numbers similar to those of the control. No cultivar effect and water regime × cultivar interactions were noted (Table 3). Data for the flooding period regarding the growth of newly-nodal roots and lateral roots are presented in terms of root length and root dry weight (Table 4). Plants subjected to flooding at ER had significant highest root traits, followed by MR and lowest at EV (Table 4). Cv. Wray showed significantly higher root traits than cv. SP1. Significant water regime × cultivar interactions were found for both root traits. Cv. Wray flooded at ER had the highest root length and root dry weight while flooded cv. Wray at EV showed the lowest root length (Fig. 4a). Cv. SP1 had the lowest root dry weight (Fig. 4b).

Aerenchyma formation

In the flooding treatments, the nodal and the lateral roots of both cultivars developed in water above the soil surface and the parts that penetrated into the soil formed aerenchyma spaces. These aerenchyma spaces were identified as lysigeneous aerenchyma. These spaces are gas spaces occurring due to the breakdown of cell walls in the cortex layers (Fig. 5). The aerenchyma spaces were also observed in the roots of the control plants, but in small amounts (data not shown). Roots that developed in water formed higher aerenchyma than those that had penetrated into the flooded soil. The nodal roots of plants flooded at EV had the highest significant aerenchyma scores, with a similar degree of development observed in the other two treatments (Table 4). The nodal roots of cv. Wray penetrated into the flooded soil and the lateral roots floating in the water had developed significantly higher aerenchyma than cv. SP1 (Table 4). The significant water regime × cultivar interaction was noted; flooding at EV giving the highest aerenchyma scores in both cultivars as well as flooding at MR of cv. SP1 while cv. Wray flooded at MR had the lowest aerenchyma score (Fig. 4c). In addition, cross-sections of sorghum stalk bases also showed the air spaces or stalk aerenchyma in the pithy areas of both control and floodedplants. The highest stalk aerenchyma was observed in plants subjected to flooding at MR, followed by ER and lowest in EV. Cv. Wray developed relatively higher stalk aerenchyma than did cv. SP1 (data not shown).

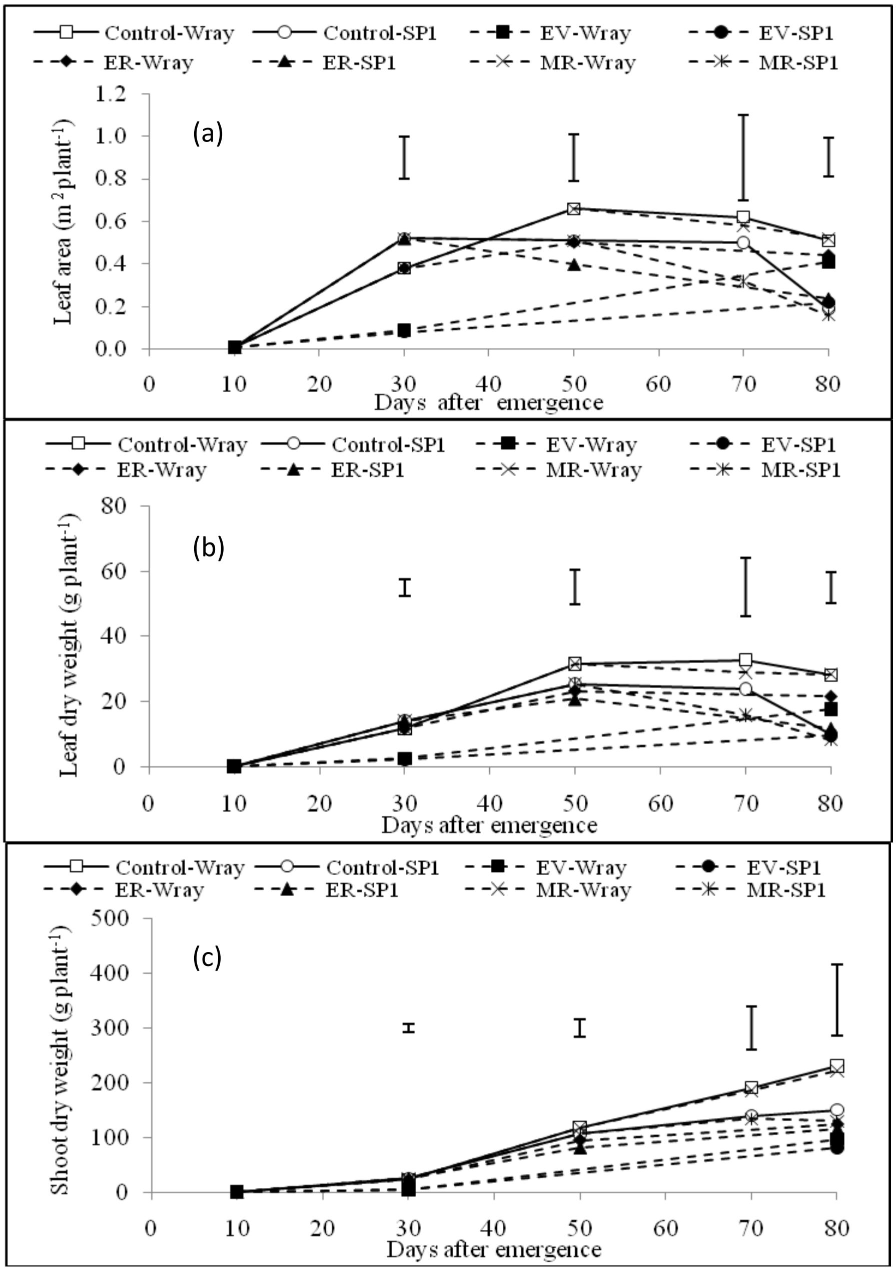


Fig 2. Change in leaf area (a), leaf dry weight (b) and shoot dry weight (c) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV: flooding at early vegetative stage; ER: flooding at early reproductive stage; MR: flooding at mid reproductive stage and control: non-flooding.

Stalk yields and Brix value responses

The stalk yields of plants subjected to flooding at MR was almost the same as those of the control. However, sorghum subjected to flooding at EV had the lowest stalk yield, 48% reduction, followed by flooding at ER, 32% reduction. Cv. Wray had a higher stalk yield (517.06 g plant^{-1}) than did cv. SP1 (247.03 g plant^{-1}) (Table 2). The significant water regime × cultivar interaction was noted for its stalk yield. Cv. SP1 flooded at EV had the lowest stalk yield. Nevertheless, as compared to its respective control, percent reduction was similar between both sorghum types (46% and 50% lower than the control in cv. SP1 and cv. Wray, respectively). When flooding was applied at ER, stalk yield was twice more than that of cv. Wray than cv. SP1, but similar reductions were found (31% and 32% for cv. SP1 and Wray, respectively).

Even though flooding at MR in both cultivars gave similar stalk yields as did the control, stalk yield was significant lower for cv. SP1 than for cv. Wray, both in terms of absolute values or reduction percentage (13% and 5%, respectively) (Table 5). This indicates that both sorghum types respond similarly when flooded at EV and ER, but multipurpose sorghum is more susceptible than sweet sorghum to flooding at the reproductive stage. Flooding had no significant effect on Brix value (total soluble solids in juice). Cv. Wray had a higher brix value (17%) than did cv. SP1 (14%). No significant water regime × cultivar interactions were noted for Brix value (data not shown).

Table 3. Effects of flooding treatments at different growth stages and cultivars on root growth of sorghum at final sampling (recovery).

Treatments	Nodal root no. per plant	Root length (m plant^{-1})	Root dry weight (g plant^{-1})
Flooding (F)			
EV	40.00b[1]	265.00c	10.54b
ER	67.63a	391.51a	23.10a
MR	72.13a	267.76bc	22.26a
Control	63.88a	342.71ab	23.32a
F-test	**	**	**
Cultivar (C)			
Wray	60.06	326.26	22.05a
SP1	61.75	307.23	17.56b
F-test	ns	ns	**
F × C			
F-test	ns	*	ns

*,** Significant at P≤0.05 and 0.01 levels, respectively and ns = not significant. [1]Means followed by the same letter are not significantly different. EV: early vegetative stage; ER: early reproductive stage; MR: mid reproductive stage and control: non-flooding.

Discussion

Our results indicate that 20 days of flooding applied at the early vegetative stage had severely reduced primary root and shoot growths the most. Flooding at the early-reproductive stage was less severe, while flooding had no significant effect at the late growth stage i.e. mid-reproductive stage. These findings confirmed the previous studies that adverse effects of flooding on crop growth and yield depends on the crop growth stage (Orchard and Jessop, 1984; Umaharan et al., 1997; Linkemer et al., 1998). Furthermore, the early growth stage is the most susceptible growth stage and crop susceptibility decreases gradually at later growth stages (Zhou and Lin, 1995; Zaidi et al., 2004). In this study, the high susceptibility to flooding at EV may be partly due to the restricted nodal root development concomitant with the findings in maize (Zaidi et al., 2004). Promkhambut et al. (2010) found that cv. SP1 is relative waterlogging tolerance. It had the ability to maintain leaf photosynthetic capacities higher than the control under waterlogging conditions at 3 leaf stage. Lertprasertrat et al. (1997) reported the similar result when germination percentage was evaluated under field conditions. In the present study, few occurrences of water regime × cultivar interactions for shoot traits at the end of each flooding treatment indicate that both sorghum types had similar shoot growth suppression. This may be due to a greater degree of root anoxia. Gilbert et al. (2007) reported similar results for sugarcane. Nevertheless, significant interactions for all shoot traits, except shoot and stalk biomass, at harvest stage indicate that sweet and multipurpose sorghum differ in recovery capacity from detrimental effects of flooding. At harvest, the cv. Wray had acquired more height, stem diameter, leaf area and leaf dry weight than had the cv. SP1. At a late growth stage, the flooded Wray had increase height and stem diameter more than the control had, but the cv. SP1 had not. This result may partly result in less stalk yield reduction when flooding was applied at MR of sweet sorghum. This finding indicates that assessing the recovery of the plant from flooding is crucial to evaluating the flooding tolerance in sorghum. For wheat (Malik et al., 2001) and barley (Pang et al., 2004), waterlogging has higher adverse effects on root growth than shoot growth. This is similar to our findings. The significant water regime × cultivar interactions in root dry weight and root length indicate that there is variation in root responses between sweet and multipurpose sorghums. In general, cv. SP1 had higher root growth under non-flooded conditions indicating that it had deeper root growth. Therefore, when subjected to flooding, root growth reduction was higher than that of cv. Wray, particularly when subjected to flooding at mid reproductive stage. Higher root length reduction of cv. SP1 when flooded at mid reproductive stage while exhibiting no significant root dry weight from control may be explained by the death of fine roots at deeper soil layers. MacFarlane et al. (2003) also found similar response in ryegrass. Pardales et al. (1991) indicated that the dieback of older roots in sorghum is concomitant with new root development in the upper node of the stalk. This shows the plasticity in the acclimation of sorghum to flooding. Nevertheless, this finding indicates that high root growth sorghum may not be suitable for production in flooding prone areas. Matsuura et al. (2005) suggested that adventitious root development during waterlogging replaces the function of dieback primary roots in waterlogged soil and plays a crucial role in supporting water and nutrient uptake in waterlogging tolerant buckwheat. These adventitious roots of flooding tolerant plants develop aerenchyma or air porosity that functions as an alternative source of oxygen supply under anaerobic conditions (McDonald et al., 2002). Pardales et al. (1991) also reported that in sorghum, nodal root development is a crucial trait for waterlogging tolerance. In sweet sorghum, Promkhambut et al. (2011) also found that nodal root number increases with the duration of flooding and plays the important role of sustaining leaf growth in flooded plants. In this study, the similar trend response of nodal root number and shoot and yield support these previous studies. The significantly highest nodal root number found at MR but lower root length indicates that the length of these new roots was restricted. This is similar to the report of Pardales et al. (1991). In addition, the observed aerenchyma development in these roots indicates that these roots could function under flooding conditions. Orchard and Jessop (1985) indicated that in grain sorghum, root aerenchyma development is an important trait giving higher waterlogging tolerance than does sunflower. The observed aerenchyma space which is higher at the vegetative stage than at the late growth stage is consistent with that found for grain sorghum, as reported by Orchard and Jessop (1985). Overall, aerenchyma scores to root cross-section area in cv. Wray were significantly larger than that in cv. SP1. There have also been reports of the positive correlation between the percentage of aerenchyma in

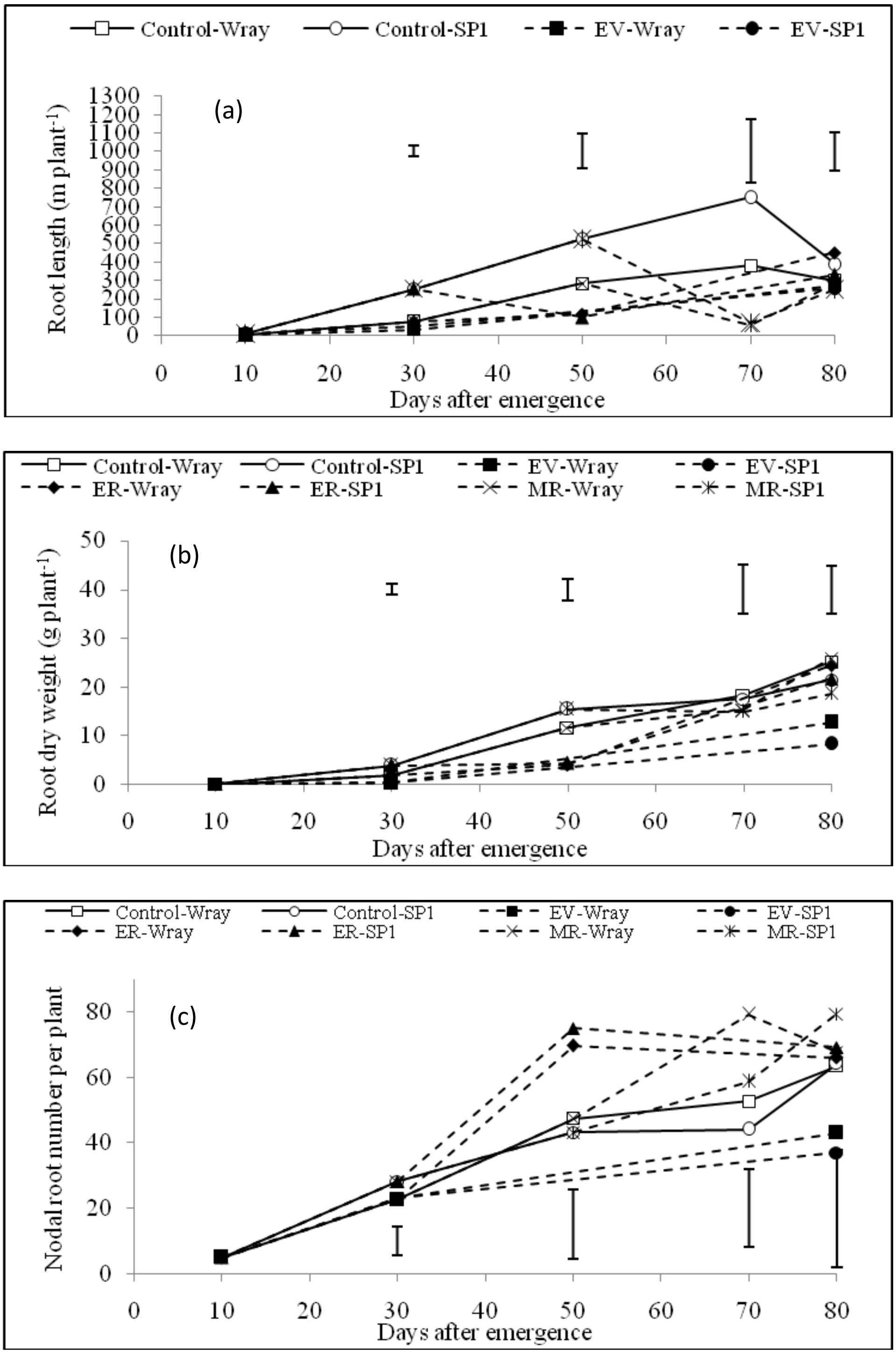


Fig 3. Change in root length (a), root dry weight (b) and nodal root number (c) for cv. Wray and cv. SP1 after 20 days of flooding at different growth stages and at harvest. EV: flooding at early vegetative stage; ER: flooding at early reproductive stage; MR: flooding at mid reproductive stage and control: non-flooding.

adventitious root and shoot growth (Huang et al., 1994) and yield (Setter and Waters, 2003) of wheat as well as higher root porosity and yield in maize (Zaidi et al., 2004). Based on this concept, cv. Wray may possess relatively higher flooding tolerance than cv. SP1. Nevertheless a high quantity of aerenchyma may be a less important flooding tolerant trait if radial oxygen is lost from the root (Setter and Waters, 2003). The significantly higher score aerenchyma in cv. SP1 flooded at mid-reproductive stage but higher stalk yield reduction may support this point of view. McDonald et al. (2002) reported low radial oxygen loss in sorghum but there is no variety comparison. Further research is needed to compare the ability to form barriers to radial oxygen loss among various sorghum genotypes. This would help to identify the genotypic possessing high flooding tolerance in sorghum. No significant water regime × cultivar interactions for nodal root number was found both at the end of flooding treatments and at final sampling. This could imply that nodal root is the general flooding root acclimation trait for sorghum, particularly under prolonged flooding. Nevertheless, relatively higher nodal root development of cv. Wray than cv. SP1 when flooding was applied at mid-reproductive stage

Table 4. Effects of flooding treatments at different growth stages and cultivars on root length, root dry weight and root aerenchyma scores after 20 days of flooding.

Flooding	Root growth[1]		Root aerenchyma scores[1]			
	Root length (m $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	NR in water	LR in water	NR in soil	LR in soil
Flooding (F)						
EV	42.84b[2]	0.39b	3.00a	3.00	2.00a	2.09
ER	171.44a	5.68a	1.63b	2.63	0.13b	1.63
MR	63.11b	1.21b	1.67b	2.75	0.06b	2.13
F-test	**	**	**	ns	**	ns
Cultivar (C)						
Wray	106.69a	2.78a	1.92	3.00a	0.96a	1.81
SP1	78.23b	2.07b	2.28	2.58b	0.50b	2.08
F-test	*	*	ns	*	*	ns
F × C						
F-test	**	*	*	ns	ns	ns

*,** Significant at P≤0.05 and 0.01 levels, respectively and ns = not significant. [1]Data are analyzed only in flooding treatments. [2]Means followed by the same letter are not significantly different. NR: nodal root and LR: lateral root. EV: early vegetative stage; ER: early reproductive stage; and MR: mid reproductive stage.

may result in the significant lower stalk yield reduction at harvest. However, based on this study's results, nodal rooting may not be a useful tool to identify genotypic flooding tolerance in sorghum. In the case of upland crop species with relatively high flooding tolerance or subjected to long-term flooding such as sugarcane, adventitious root development may also not be a flooding tolerant screening tool (Gilbert et al., 2007). Gilbert et al. (2007) indicated that stalk aerenchyma is an important indicator for differentiating genotypic flooding tolerance in sugarcane. They found that cultivar with constitutive stalk aerenchyma had higher flooding tolerance. The observed higher aerenchyma development in stalk bases of cv. Wray than cv. SP1 may be one of the important flooding tolerant traits in sweet sorghum. However, in this experiment, the study in this trait was only preliminary and used only two cultivars. Further research should concentrate on this particular trait with a larger number of genotypes. Significantly different water regime × cultivar interactions for root development in water indicate that the growth stage of a plant is important for the study on root acclimation to flooding. Significant cultivar differences occurred when flooding was applied at early-reproductive stage but not at early vegetative and mid-reproductive stages, indicating that morphological root acclimation traits may not be useful flooding tolerant indicators at too early and too late a growth stage. Umaharan et al. (1997) reported that cowpea waterlogged at its reproductive stage lost its root acclimation ability.

They mentioned that ability to maintain leaf area and leaf growth are more important than root acclimation. In grain sorghum, Ali et al. (2011) reported that different morpho-physiological traits should be used to classify drought tolerance among sorghum genotypes at different growth stages. They found that root and shoot growth parameters were a reliable technique for screening genotypes for drought tolerance at an early growth stage. On the other hand, leaf growth parameters were useful markers contributing towards drought tolerance during times of terminal drought. With respect to this concept, lower leaf area and leaf dry weight reduction than the control of cv. Wray compared to cv. SP1 when flooding was applied at mid reproductive stage may imply that sweet sorghum is more tolerant to flooding at a later growth stage than is multipurpose sorghum. This result may be one of the factors supporting the high stalk yield of sweet sorghum. Nevertheless, the relative higher grain yield of cv. SP1 may cause competition of photosynthates with stalk when flooding was applied at the mid- reproductive stage, which was concurrent with the grain-filling stage. Beheshti and Behboodifard (2010) found that under drought conditions, grain sorghum exposed to water deficit during grain filling tends to utilize stored assimilates from other parts by increasing the amount of remobilized dry matter, the remobilization efficiency and the remobilization percentage. This consequently resulted in decreased stem weight. On the other hand, there is no competition in sweet sorghum between grain development and sugar accumulation in the stalk (Lingle, 1987). Under pre-rice crop production conditions, flooding often occurs at mid-rainy season due to rainfall intensity and rising water table level. The early rains during the rainy season could be successfully used to grow sweet sorghum by selecting an early planting date to avoid the early growth stage. Therefore, sweet sorghum is preferable to multipurpose sorghum for this cropping system. In addition to flooding/waterlogging constraints during the pre-rice growing period, intermittent water stress is also a prevalent problem (Lantican, 1982). Zaidi et al. (2008) reported that there are relationships between drought and excess moisture tolerance in tropical maize (*Zea may*s L.). Further research evaluating the response of sweet sorghum under drought and flooding/waterlogging conditions might improve the yield under pre-rice growing conditions. No significant difference in juice quality, as indicated by Brix value between flooding and control plants in both sorghum types, show that sucrose synthesis and allocation was unaffected by flooding. This is similar to the response of sweet sorghum to drought stress (Massacci et al., 1996) and flooding conditions (Promkhambut et al., 2011) as well as sugarcane response to flooding (Gilbert et al., 2007).

In conclusion, both sorghum types are susceptible to flooding at early-vegetative stage and the susceptibility is decreased at a late growth stage. Nodal root development plays a crucial role in flooding tolerance for both sorghum types. However, this root trait may not be a useful trait to identify genotypic flooding variation under severe flooding or relative flooding tolerant genotype. Aerenchyma development in roots and stalk may be more important traits. Cv. Wray showed significantly higher flooding tolerance based on stalk fresh weight than cv. SP1 when flooding was applied at late growth stage. It also had the capacity of the recovery of plant height and stem diameter, relative higher nodal root number and aerenchyma formation in root during flooding and observed higher stalk aerenchyma.

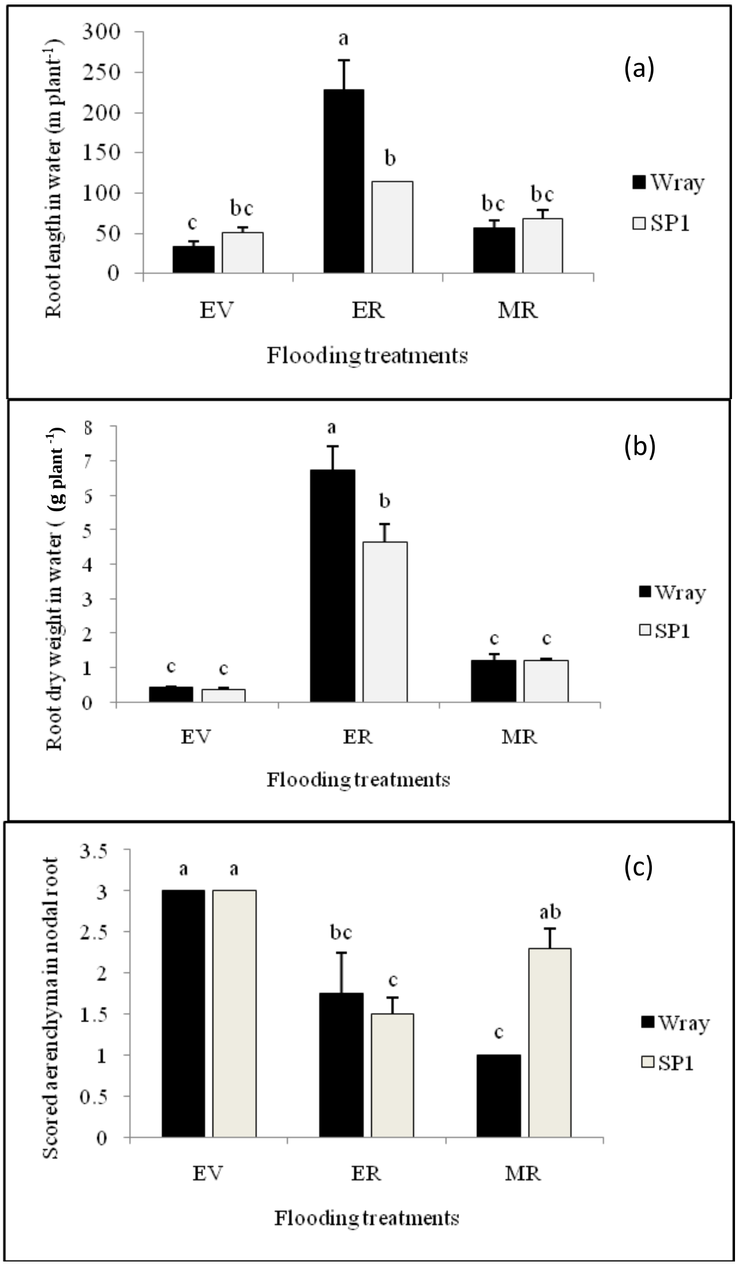


Fig 4. Root growth parameters developed in standing water after 20 days of flooding, root length ($p \leq 0.01$) (a), and root dry weight ($p \leq 0.05$) (b) and scored aerenchyma of nodal roots developed in water $P<=0.05$ (P less than or equal 0.05) (c). EV: flooding at early vegetative stage; ER: flooding at early reproductive stage and MR: flooding at mid-reproductive.

This indicates that cv. Wray is more suitable for pre-rice crop production conditions. In addition, as more than twice as much higher stalk yield in sweet sorghum than in multipurpose sorghum, sweet sorghum production in paddy fields is recommended. Moreover, early sowing to avoid growth stages that are susceptible to flooding is the most economical solution for energy crop production in paddy fields.

Materials and methods

Plant materials and culture

The experiment was conducted from May to August 2005 under greenhouse conditions with natural sunlight and photoperiods at Agronomy Field, the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, KhonKaen University, Thailand (16°26'N 102°50' E above sea level 204 m). Pots (38 cm height, 37.5 cm wide) were arranged 15 cm apart. Each pot was filled with 30 kg of sieved air-dried loamy soil (taken from irrigated paddy fields in Thailand) containing 2.18% organic matter, 5.05 ppm of available phosphorus and 159.5 ppm extractable potassium. Fertilizer was thoroughly mixed in the soil of each pot to provide the equivalent of 47 kg ha^{-1} of N, P_2O_5 and K_2O. Each pot was then watered to approximate field capacity. This moisture content was maintained by weighing the pots every other day using a scale platform balance. Into each pot, seeds of *S. bicolor* cv. Wray, sweet cultivar and cv. Supanburi 1, SP1, multipurpose cultivar, were sown. Four to

Table 5. Stalk yield (stalk fresh weight, g plant^{-1}) of sweet and forage sorghum at final sampling.

Flooding	Stalk yield (g plant^{-1})			
	EV	ER	MR	Control
Wray	333.13c[1](50)	448.05b(32)	627.05a(5)	660.00 a
SP1	172.65e(46)	219.25de(31)	278.00cd(13)	318.23c

Figures in parenthesis indicate percent decrease as compared to the control. [1]Means followed by the same letter are not significantly different at 0.01 probability level. EV: early vegetative stage; ER: early reproductive stage; MR: mid reproductive stage and control: non-flooding.

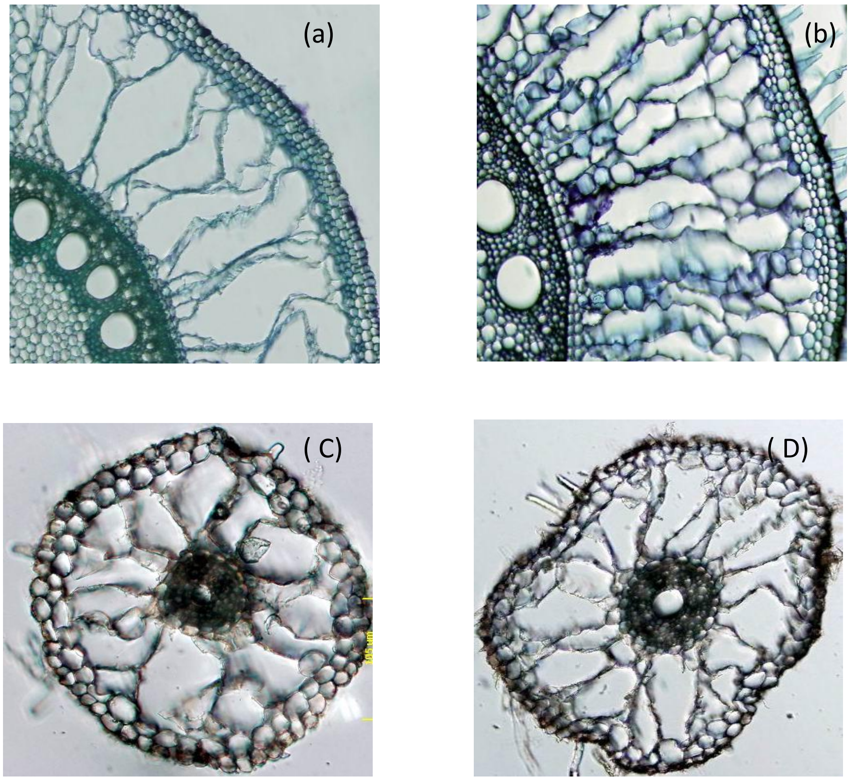


Fig 5. Cortical aerenchyma in nodal root (NR) and lateral root (LR) that penetrated in flooded soil of cv. Wray (a and c) and cv. SP1 (b and d) when 20 days of flooding was applied at early vegetative growth stage. AC: aerenchyma space. Bar= 105 µm.

five seedlings were initially planted per pot but these were thinned to one plant per pot at 7 days after emergence (DAE). Cv. Wray is reported to be a superior source of sugars along with good agronomic features (Ferraris, 1981), while cv. SP1 is grown for its grain and its stem for animal feeding. In addition to its high sugar content in the stem, compatibility with Thai growing conditions and its relative waterlogging tolerance (Lertprasertrat et al., 1997), this particular cultivar could be suitable for areas prone to flooding.

Experimental treatments

The experimental treatments consisted of four flooding treatments and two cultivars arranged in a factorial experiment in a randomized complete block design with 4 replications. The 20 days of flooding treatments were applied at (i) early vegetative stage, 10 DAE (EV), (ii) early reproductive stage, 30 DAE (ER) and (iii) mid reproductive stage, 50 DAE (MR) **(**Vanderlip and Reeves, 1972)**.** During flooding periods, the water level was raised to 3 cm above the soil's surface by adding water to each pot daily. At the termination of each treatment, each pot was drained by making a hole at the bottom. In the control treatment (CK), soil moisture was kept at field capacity during the whole growth period. Pesticides and insecticides were used as needed.

Shoot and root growth analysis

Each treatment had three sets of three samplings per set. The first sampling was taken one day before flooding. The second sampling was taken at the end of each flooding treatment, and the third samples were taken after plant recovery from flooding at 80 DAE (i.e. 50, 30 and 10 days after the end of flooding in the three respective treatments). Plants were cut at the soil surface and samples were separated into main stems and tillers. These were further divided into head, culm (stem and leaf sheath) and leaf components. Plant height was measured from the soil surface to the top of the leaf during the early reproductive stages and to the top of the head following panicle emergence. Stem diameter at first node was measured. Leaf area was measured by automatic leaf area meter, ACC-400 (Hayashi-Denko co, Ltd., Tokyo, Japan). Shoot material was dried at 80 °C for 48 hours to determine dry weight. After the shoots were harvested, roots were carefully washed from the soil over a sieve (mesh, 2 mm). The nodal root (NR) number per plant was counted. Roots were divided into those standing above soil level (standing water) and below soil level (flooded soil). Roots were scanned and total root length from each pot was measured by analyzing pictures using WinRHIZO pro V2004a (Reagent Instruments Inc., USA). Roots were dried at 80 °C for 48 hours to determine dry weight.

Aerenchyma observation

Twenty days after flooding for each treatment, root anatomy was examined in nodal and 1st order lateral roots of flooded plants in both water above soil surface and soil below ground surface. Roots of control plants were similarly examined. Segments of nodal roots were dissected from the 2nd and 3rd flooded nodes from the top at 3-5 cm away from the root-shoot junction, whilst the 1st order later root was sampled at 10-20 mm from that nodal root segment. Root segments in flooded soil were dissected 2-3 cm below the soil surface. Nodal roots and 1st order lateral roots were sampled in the same way for roots in stagnant water. The tissues were fixed in 70% FAA (formalin, acetic acid, 70 % ethanol; 1:1: 18 parts by volume) according to Pardaless et al. (1991). Free hand cross-sections of the sections stained by toluidine blue (0.01%) were observed using an Olympus biological microscope CX3 with a 4x and 10x objective lens and digitally imaged by an Olympus microscope digital camera system DP-12. The amount of aerenchyma in the root cortex was visually scored using the system of Mano et al. (2006). The amount of aerenchyma in the root cortex was visually scored using the following designations: 0= no aerenchyma, 0.5= partial formation, 1= radial formation, 2= radial formation extendedtowardepidermis and 3= well-formed aerenchyma. Moreover, at harvest, stalk bases of the first nodes of plants were transversely cross-sectioned to observe the air spaces or stalk aerenchyma in their pithy areas.

Yield and quality analysis

At the final harvest, the stalks of sorghum (above the soil surface to the uppermost internodes) were stripped and weighed to obtain the yields. After that, juice was extracted using crushing equipment and the Brix value (soluble solid, %) of the juice was measured by hand digital refractometer PAL-1 (ATAGO, Japan).

Data analysis

Analyses of variances for all collected data were performed using Statistix 8 software (Analytical Software, Tallassee, USA). To analyse the significant differences of treatment effects, the data set was partitioned into separated growth stages of each flooding treatment and the harvest stage (recovery) with replications as random effects, and the cultivar and flooding as fixed effects. The Least Significant Difference test was used to evaluate the differences between the means of the treatments.

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